Forest thinning has little effect on spatiotemporal dynamics of arbuscular mycorrhizal fungi community in *Cryptomeria japonica* roots

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Abstract

Stand thinning affects forest physiognomy above- and belowground, but we ignore how it affects root-associated arbuscular mycorrhizal fungi (AMF) in trees. We aimed to investigate how stand thinning affects the dynamics of the AMF community in trees. Root and soil samples of twenty selected *Cryptomeria japonica* (Cupressaceae) trees were collected every August from 2021 to 2023 at four microsites with and without stand thinning, established in nearly 1 km^2 of a *C. japonica* plantation in central Japan. We amplified ~550 bp of a partial small subunit of fungal ribosomal DNA and amplicons were sequenced with Illumina Miseq to investigate the root AMF community composition. Soil pH, total C, N, and C/N were also measured. As a result, we observed significant (1) spatial variation in pH, total C, and N; (2) spatiotemporal dynamics in C/N, AMF richness, and Shannon index increasing from the first year to the second, then decreasing down to the initial status from the second year to the third; and (3) spatial variation in the AMF community composition mainly driven by soil pH, total C, N, and C/N; all irrespective of stand-thinning treatment. A light thinning does not suddenly affect the soil properties that influence AMF distribution, and thus the root AMF community of spared trees remains unchanged till two years post-thinning. Our findings warned that stochasticity should be considered when analyzing AMF's response to treatments in long-term studies.

Keywords: Forest management, Thinning, AMF ecology, Metabarcoding, Spatiotemporal distribution

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Introduction

Thinning is a silvicultural operation that affects the physiognomy of forests, from aboveground to belowground. In the aboveground, thinning opens the canopy area for remaining trees and affects the native herbaceous and shrub plant communities (Overby et al. 2015; Dang et al. 2018; Caihong et al. 2023; Liu et al. 2023a). In the belowground, thinning increases the concentrations of soil organic carbon (C), total nitrogen (N), total phosphorus, nitrate nitrogen, and available phosphorus (Dang et al. 2018; Caihong et al. 2023; Liu et al. 2023a) and affects fungal community including arbuscular mycorrhizal fungi (AMF) in the soil (Maassen et al. 2006; Owen et al. 2009; Bach et al. 2010; Overby et al. 2015; Dang et al. 2018; Lei et al. 2021; Caihong et al. 2023). However, its effects on the AMF community in the roots of spared trees over time and space remain unclear, and thus a significant gap of knowledge in AMF community ecology regarding forest management and ecology. In this study, we aimed to investigate how stand thinning spatially and temporarily affects the AMF community in the roots of spared trees.

Ecological disturbances to AMF communities may originate from mechanical soil disturbance, agricultural activities, host disturbance, and chemical application resulting in the disruption of hyphal networks and a fertilization-related erosion of biological diversity (Amalia et al. 2021). Mechanical soil disturbances induce significant decrease and increase in the richness of operational taxonomic units (OTUs) of AMF, a measure of species richness, and significant compositional changes in the AMF community (Schnoor et al. 2011; Kawahara and Ezawa 2013; Atunnisa and Ezawa 2019; Liu et al. 2023b). As for the host disturbance, it may (Sharmah and Jha 2014; Liu et al. 2023b) or may not (Lekberg et al. 2012) significantly affect AMF species richness and community compositions. Major disturbances such as forest clear-cutting may affect soil AMF inocula in species-specific manners due to the different response of different AMF taxa to the loss of vegetation or the other previously mentioned types of disturbances (Hart and Reader 2004; Cahyaningtyas and Ezawa 2023). Thus, thinning boosts wood production in planted forests, but may constitute ecological disturbances to plant and microbial communities above and belowground. Root colonizing AMF communities may be highly tolerant to ecological disturbances besides the substantial local stochasticity (Lekberg et al. 2012; Cahyaningtyas and Ezawa 2023), but it was found that hurricane strongly impacts on root AMF community in a forest by changing its network structure (Alvarez-Manjarrez et al. 2024). Therefore, the effects of natural and artificial disturbances on an AMF community may depend on the host plant, the type and intensity of disturbances, and the biology of the mainly colonizing AMF. Still, the effect of forest thinning on the root-colonizing AMF of the undisturbed trees remains unclear.

In forest ecosystems, multiple AMF co-colonize multiple plants, forming complex mycorrhizal networks (Bunn et al. 2024). Thus, the removal of some trees during thinning may not affect the established mycorrhizal networks unless significant mechanical soil disturbances occurred. In this regard, the composition of the root AMF community of spared trees will remain undisturbed. On the other hand, co-occurring AMF in the same root system respond differently to soil properties such as pH, showing negative correlations to each other in response to variations in pH (Djotan et al. 2024a), emphasizing the realized

niche space occupied by individual and phylogenetic groups of AMF (Davison et al. 2021). Thus, even without significant mechanical disturbances to the soil, thinning could indirectly but significantly change the root AMF community composition of spared trees if its implementation changes soil physicochemical properties significantly. Therefore, we can expect that stand thinning effects on the root AMF community of spared trees through changes in the soil physicochemical properties.

Ecological studies of AMF associated with *Cryptomeria japonica* (Cupressaceae), the topmost planted tree in Japan and also planted in Taiwan and China, using molecular methods have revealed a higher prevalence of AMF in first-order fine roots than in second- and third-order roots (Matsuda et al. 2021), a nestedness pattern of root AMF community in that of the surrounding soil (Djotan et al. 2022), a differential exploration of root and soil among AMF taxa (Djotan et al. 2023), seasonal shifts between root and soil of root colonizing AMF (Djotan et al. 2024b), and spatiality and cohabitation of two to four co-dominant AMF in the same root system (Djotan et al. 2024a). These previous studies provided significant insights into the AMF community ecology in forest ecosystems, but none has covered the ecology of AMF community ecology regarding forest management which constitutes an ecological disturbance. Also, the interannual dynamics of AMF regardless of the target ecosystems remain a significant gap of knowledge. Thus, in the current study, we investigated selected trees at microsites with and without thinning in a plantation of *C. japonica* in central Japan for three consecutive years to investigate how stand thinning spatially and temporarily affects AMF community in the roots of spared trees. Because previous studies have found that thinning of *C. japonica* plantation does not significantly affect the soil physicochemical properties in the short-term (Yamada et al. 2015; 2016), we hypothesized that stand thinning does not significantly affect the root AMF community of remaining trees.

Materials and methods

Study site and sampling design

The study site was a planted forest of *C. japonica* (177 ha) in the cool-temperate region of Japan (Saitama Prefecture; 35.94 °N, 138.83 °E) where the surrounding vegetation was a naturally regenerated forest composed of deciduous hardwoods. The mean annual temperature and mean annual precipitation are 11.6 °C (2014 – 2023) and 1,420.8 mm (2014 – 2023), respectively, based on data from Tochimoto station (35.94 °N, 138.86 °E, 760 m a.s.l.) of the University of Tokyo Chichibu Forest. The understory vegetation is mainly composed of *Amphicarpaea edgeworthii* (Fabaceae), *Chloranthus serratus* (Chloranthaceae), and *Viola tokubuchiana* var. *takedana* (Violaceae) (Djotan et al. 2023).

Every August from 2021 to 2023, we collected roots and surrounding soils of *C. japonica* trees at four microsites (MSs 1, 2, 4, and 5) in permanent plots previously established at UTCF (Djotan et al. 2024a). The microsites were established along two topographic profiles, where MSs 1 and 4 were located at elevations of approximately 1,030–1,045 m a.s.l. (Online Resource 1, Table S1). MS2 (same topographic profile as MS1) and MS5 (same topographic profile as MS4) were located at elevations of approximately 900 m a.s.l.. Stands of *C. japonica* at MS1 and MS4 were not thinned during the three-year survey, but after the sampling in August 2021, thinning at a 30% intensity was applied to the stands at MS2 and MS5.

Closest and farthest microstes were 130 m and 250 m apart, respectively. At each microsite, five trees of *C. japonica* were arbitrarily selected. A basal root of each tree was traced from its base and sampled together with the surrounding soil as a buffer without disturbing the first- and second-order roots (Djotan et al. 2022). In total, 60 paired root–soil samples were collected over three years from four microsites with and without thinning.

Soil physicochemical properties

We air-dried soil samples and passed them through a 500-μm mesh sieve before physicochemical analysis. We added 50 mL of sterilized distilled water to 20 g of air-dried soil and shook it for five minutes. The mixtures were allowed to stand for 30 min and the pH was measured from 500 μL of the supernatant using a compact pH meter (LAQUAtwin-pH-33; Horiba, Kyoto, Japan). We powdered 20 mg of air-dried soil and dry-combusted them in a Sumigraph NC-80 Analyzer (Sumika Chemical Analysis Service, Co., Tokyo, Japan) following the manufacturer's instructions to analyze total C, and N contents.

DNA extraction and amplification

We lyophilized and milled root samples before getting them DNA-extracted using a DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. We confirmed the identity of processed root samples by amplifying approximatively 550 bp of the partial rubilose biphosphate carboxylase large subunit (*rbc*L) region using the primers rbcLaF (5′-ATG TCA CCA CAA ACA GAG ACT AAA GC-3′) (Hasebe et al. 1994) and rbcLaR (5′- GTA AAA TCA AGT CCA CCR CG-3′) (Ferri et al. 2015). Polymerase chain reaction (PCR) for the amplification of approximately 550 bp of the fungal small subunit ribosomal fungal DNA (SSU rDNA) was performed on the identity-confirmed samples. We used the primer set AML1 / AML2 (Lee et al. 2008) in the first-round PCR and the primer set NS31 / AM1 (Simon et al. 1992; Helgason et al. 1998) in the second-round PCR, as described by Djotan et al. (2024b). The Illumina adapters Tn5ME A and Tn5ME B were linked to the primer set NS31/AM1, and the purified PCR products were multiplexed by pools of five samples. After purification, the amplicons of *rbc*L and SSU rDNA were sent to Macrogen Japan (Tokyo, Japan) for Sanger (~550 bp) and Illumina MiSeq (2 × 300 bp) amplicon sequencing, respectively.

Bioinformatic and phylogenetic analyses

To confirm that the processed root samples contained *C. japonica* as expected, we visualized the amplicon sequences of the *rbc*L gene for quality control in BioEdit computer program (Ibis Biosciences, Carlsbad, CA, USA) and BLASTed against the National Center for Biotechnology Information (NCBI) GenBank database. We processed the Illumina MiSeq amplicon sequences using QIIME 2 v. 2022.2.0 (Bolyen et al. 2019). Briefly, we demultiplexed the reads and merged the pairs to discard the forward and reverse reads that could not be merged successfully. In the merging, we allowed an overlap of at least 10 bp and merged sequence sizes between 500 and 600 bp. Next, we filtered the merged sequences based on the q-score over a quality window of 3 bp to keep those with a Phred quality score ≥ 20, and a minimum length fraction of 0.75. We clustered the resulting sequences into operational taxonomic units (OTUs) at a 97% sequence similarity threshold, excluding rare (< 10 reads across all samples or detected in only one

sample) and chimeric OTUs. We assigned taxonomic information, which we updated following the consensus AMF classification (Redecker et al. 2013), to the retained OTUs by locally BLASTing them against the Maarj*AM* (Öpik et al. 2010) and NCBI GenBank databases using the NCBI-blast-2.10.0 + program (Morgulis et al. 2008). We deposited the sequence read archives at the NCBI (PRJNA898865). The representative nucleotide sequences of the AMF OTUs generated (OR744065– OR744740, SUB13933440) and a representative partial nucleotide sequence of *rbc*L for *C. japonica* (OP832014, BankIt 2642437) were submitted to GenBank.

The OTUs with an average relative abundance > 1% at the study site were defined as major OTUs and those with an average relative abundance of at least 10% at the site or a microsite were defined as the dominant OTUs associated with the host plant. We aligned the representative sequences of the major OTUs using MAFFT v7.490 (Katoh and Standley 2013). Their phylogenetic positions were inferred using an automatic model finder in IQ-TREE 2 (Minh et al. 2020).

Statistical analysis

We performed all statistical analyses using R v.4.3.2 with a confidence level of 0.05 (R Core Team 2023). Spatiotemporal variations in soil physicochemical properties were tested using a two-way (two-factor) mixed analysis of variance (ANOVA) for repeated measures (Schober and Vetter 2018), involving microsite as the between-subjects factor (analysis of spatial variation) and sampling year as the within-subjects factor (analysis of temporal variation related to thinning disturbance). Before computing the mixed ANOVA test using the R function *anova_test*() [rstatix package], we performed preliminary tests to validate the assumptions for this parametric test. Normal distribution, homogeneity of variances, homogeneity of covariances, and sphericity were validated using the Shapiro test, Levene test, Box's M-test, and Mauchly's test, respectively. Mauchly's test was internally used to assess the sphericity assumption. Then, Greenhouse-Geisser sphericity correction was automatically applied to factors violating the sphericity assumption. We performed multiple pairwise t-tests where *p*-values were adjusted using the Bonferroni multiple-testing correction method to identify different groups. The spatiotemporal variations (between the microsites, the sampling years, and their interaction) in the OTU richness and Shannon index of the AMF community were tested as described previously. The AMF community data was normalized to the relative abundance of OTUs in each sample and ordinated using a non-metric multidimensional scaling (NMDS) on the Bray–Curtis dissimilarity matrix. We visualized the ordination to analyze potential variations between microsites and sampling years, which were tested with a two-way nested permutational ANOVA (PERMANOVA) using *adonis2* of the vegan package. Multiple pairwise PERMANOVA in *adonis2* was used to identify significantly different AMF communities among levels of factors with significant effects. We computed the average relative abundance of each OTU at each microsite (n = 5) and averaged the microsites' means to obtain the average relative abundance at the study site (n = 4). Based on these values for the major OTUs (including dominant OTUs), we analyzed the effects of thinning disturbance on the root AMF communities of *C. japonica* across microsites. The relationships between the relative abundance of dominant OTUs and the physicochemical environment were also analyzed using redundancy analysis

(RDA) tested with PERMANOVA. The correlation between soil's physicochemical properties and their associated *p*-value were computed with envFit of the vegan package.

Results

Sequencing summary

We obtained 1,096,309 amplicon sequences, which were clustered into 12,446 OTUs at 97% sequence similarity with more than 2,393 reads per sample (the maximum was 38,454) after trimming, pair joining, and quality filtering. After removing chimeric OTUs, non-Glomeromycotina OTUs, and rare OTUs, 971,582 sequences remained and clustered into 676 OTUs, with the minimum and maximum frequencies per sample being 2,260 and 33,485, respectively. The OTU accumulation curves showed that the number of samples and the number of sequences included in the analysis of the AMF community were sufficient to reveal its diversity and composition at the study site (Online Resource 2).

Soil physicochemical properties

The soil pH, total C, N, and C/N were significantly different ($p < 0.01$) between microsites, but only C/N was significantly different ($p = 0.03$) between sampling years (Tables 1 and S2). Microsite showed the biggest effect size and the interaction between microsite and sampling year was not significant. pH at MS1 was significantly higher than that at the others; total C and N at MS1 and MS2 were not significantly different but significantly lower than that at MS4 and MS5 which were not significantly different; for C/N, MS2 was significantly different with the lowest value compared to the others (Table S3).

AMF community composition of *C. japonica* roots with special reference to stand thinning

We detected in total 577, 625, and 562 OTUs in 2021, 2022, and 2023, respectively (Online Resource 2). The average number of OTUs and Shannon index were significantly different between microsites and sampling years, but without a significant interaction (Tables 2 and S4). They increased from 2021 before thinning to 2022 after thinning and then decreased to the same level in 2023 as before the thinning (Online Resource 2, Table S5). This pattern was observed at microsite with and without a thinning treatment.

The composition of the AMF community in the roots of *C. japonica* was significantly different between microsites ($p < 0.001$) but not between sampling years ($p = 0.07$) (Fig. 1, Table S6). *Cryptomeria japonica* hosted a unique AMF community in its roots at each microsite but the dissimilarity between the AMF communities at MS1 and MS5 was the least compared to other pairs of microsites (Table S7). We detected 14 major OTUs of which nine were dominant (Table 3). The phylogenetic analysis showed that four of the major OTUs (OR744066, OR744070, OR744072, and OR744078) belong to unknown or unresolved genera of Glomeraceae (Online Resource 3, Table 3). The others were placed into five genera: *Dominikia* (2 OTUs), *Glomus* (2), *Microkamienskia* (1), *Rhizophagus* (4), and *Septoglomus* (1).

The variation in the AMF community of *C. japonica* by microsite was explained differently by soil pH, total C, N, and C/N (Fig. 2, Table S8). The total C and N contents significantly ordinated the community following the first axis of the RDA (RDA1: 50.43%, $p = 0.001$). These two soil properties showed positive correlation with the dominant OTU OR744066 (unresolved Glomeraceae). The soil pH significantly

ordinated the community following the second axis of the RDA (RDA2: 28.91%, p = 0.002). It positively correlated with the dominant OTU OR744065 (*Dominikia*). As for the C/N, it ordinated the community following both axes and negatively correlated with the dominant OTU OR744067 (*Microkamienskia*).

Fig. 1 Non-metric multidimensional scaling of the arbuscular mycorrhizal fungi (AMF) community in *Cryptomeria japonica* roots at four microsites with and without thinning treatment. Stress = 0.20. Points represent samples, their color indicates the microsite, and their shape represents the sampling year. Thinning was applied in 2021 after sampling at MS2 and MS5 (dotted lines) but not at MS1 and MS4 (plain line). The AMF community was significantly different between microsites (PERMANOVA, p = 0.001) but not between sampling years at each microsite (PERMANOVA, p = 0.07)

Fig. 2 Triplot of redundancy analysis (RDA) of the arbuscular mycorrhizal fungi (AMF) community in the roots of *Cryptomeria japonica*, visualized on scaling 2. Asterisks following the names of vectors indicate a significant correlation with the AMF community composition (***, p < 0.001; **, p < 0.01; *, p < 0.05). OR###### are the accessions of the three operational taxonomic units (OTUs) most influenced by soil's physicochemical properties, explaining the variation observed in the AMF community; MS, microsite; TC, total C; TN, total N; C/N, carbon to nitrogen ratio

Table 1 Soil physicochemical properties at four microsites established in an artificial *Cryptomeria japonica* forest

Five *C. japonica* trees were selected at each microsite. Values are presented as average (n = 5) ± SD for pH, total C, N, and C/N (carbon to nitrogen ratio). The interaction between microsite and sampling year was not significant (two-way mixed analysis of variance, p > 0.05). At a confidence level of 0.05, these soil's physicochemical properties were significantly different between microsites but not between sampling years, except for C/N (see Tables S2 and S3)

Table 2 Alpha diversity of the arbuscular mycorrhizal fungi communities *Cryptomeria japonica* roots before and after stand thinning

Five *C. japonica* trees were selected at each microsite. Values are presented as average (n = 5) ± SD. The interaction between microsite and sampling year was not significant (two-way mixed analysis of variance, p > 0.05). At a confidence level of 0.05, the number of OTUs and Shannon index were significantly different between microsites and sampling years (see Tables S4 and S5)

Table 3 Distribution of the major operational taxonomic units of arbuscular mycorrhizal fungi in the roots of *Cryptomeria japonica* before and after stand thinning

OTUs, operational taxonomic units; MS, microsite. Dominant OTUs are marked with "*" and correspond to those with more than 10% of average relative abundance at one or more MS

Discussion

We hypothesized that stand thinning does not significantly affect the root AMF community of remaining trees. As expected, we found that the short-term temporal effects (two years post thinning) of 30% thinning intensity of the investigated *C. japonica* plantation did not significantly affect the soil physicochemical properties, supporting Yamada et al. (2015; 2016). In line with our hypothesis, the applied stand thinning (30% of intensity) in the artificial forest of *C. japonica* did not affect the AMF community of the spared trees. Thus, trees that remain after a low intensity of thinning conserves their root AMF community in the short term, till two years post thinning.

We found that variations in the soil physicochemical properties were significant between microsites (spatial effect). As for the variation between sampling years (temporal effect), only C/N was significantly varied. However, the interaction between microsite and sampling year was not significant and the variation of C/N between sampling years occurred independently of thinning treatment. Consequently, the detected temporal variation of C/N was introduced by the strong variation of soil physicochemical properties between microsites. Thus, the applied thinning intensity of 30% was not disturbing enough to effect on the physicochemical properties of the soil within two years post-thinning of *C. japonica* plantations (short-term temporal variation). In the long term, the effects of thinning on the soil properties due to changes in litter decomposition and nutrient status may appear (Overby et al. 2015; Zhou et al. 2016; Dang et al. 2018; Liu et al. 2023a). However, in the short term, such changes could not be detected in the current study. Similarly to our finding, even with thinning intensities ranging from 0% to 50% in a Chinese fir (*Cunninghamia lanceolata*) plantation, a short-term (two years post thinning) variation in soil physicochemical properties was not significant (Xu et al. 2020).

The effects of thinning on soil fungal communities including AMF were investigated in previous studies (Maassen et al. 2006; Owen et al. 2009; Bach et al. 2010; Overby et al. 2015; Dang et al. 2018; Lei et al. 2021) but our study was the first to investigate the effects of thinning on the root AMF community of spared trees after thinning. In our study, the detected AMF community in the roots of *C. japonica* had its OTU richness and Shannon index varied between sampling years independently of thinning treatment. They increased from 2021 before thinning to 2022 after thinning and then decreased to the same level in 2023 as before the thinning. Meanwhile, the community composition was not significantly different between sampling years. These results suggest that different mechanisms drive the temporal dynamics in the alpha and betta diversity of AMF community. It is known that both stochastic and deterministic changes occur over time in AMF community (Dumbrell et al. 2010). Our data showed that alpha diversity (measured by OTU richness and Shannon index) is susceptible to stochasticity (temporal variation) while beta diversity (measured by community composition) is susceptible to deterministic processes (spatial variation among microsites explained by soil pH, total C, N, and C/N). Deterministic processes where soil pH, total C, N, and C/N drive the structure of *C. japonica* associated AMF community have been previously reported (Djotan et al. 2024a, b). Here, the observed interannual dynamics in the alpha diversity of the root AMF community warns that the analysis of the effects of given treatments to the response of AMF community should consider the interannual stochasticity if multiple-years experiments are conducted. Among studies tackling

the effects of host disturbance on AMF community, some found that it does not significantly affect AMF species richness and community compositions (Lekberg et al. 2012; Xu et al. 2020) while others found that it does (Sharmah and Jha 2014; Liu et al. 2023b). Stochasticity must have had important noises explaining these opposing results. Meanwhile, the effects of host plant species and study site cannot be overlooked.

We observed a niche specialization of three dominant AMF in the roots of *C. japonica* driven by soil pH, total C, N, and C/N. The OTU OR744065 (*Dominikia*) was positively and significantly correlated with pH; OR744066 (unresolved Glomeraceae) positively and significantly correlated with total C and N; and OR744067 (*Microkamienskia*) was negatively and significantly correlated with C/N which also affect the distribution of the two other dominants OTUs. Based on these relationships, we speculate that *C. japonica* refine its root colonization based on the soil's physicochemical properties. The AMF that sustains the symbiosis in each soil microenvironment settles in the roots of the host plants. This is supported by the distribution of the three mentioned dominant OTUs in the roots of *C. japonica* over space and time. These results corroborate with the findings that different AMF respond differently to environmental factors and closely related AMF exhibit similar niche optima and widths (Davison et al. 2021; Xu et al. 2022; Djotan et al. 2024a). Although the current study cannot ascertain whether they are complementary or interfere with one another, we suggest that a good consortium of AMF for growing seedlings of *C. japonica* must contain them. The findings of the current study highlight how soil pH, total C, N, and C/N can be manipulated to isolate the most abundant AMFs in the roots of *C. japonica* using pot trap cultures. If isolated, the interactions between these AMF and the host plant will clarify AMF assembly mechanisms in trees and how many AMF can simultaneously colonize the same root system.

In conclusion, stand thinning does not affect AMF community in the roots of spared trees within two years post thinning when the thinning intensity does not affect the soil's physicochemical properties that drive AMF community assembly. Our findings stressed that a thinning that conserves intraradical AMF community is possible, which we encourage forest managers to practice for simultaneously achieving a good forest productivity and a healthy soil for an ecological balance. On the other hand, regardless of thinning disturbances, there is an important stochastic interannual variation of AMF community, which it is important to consider when designing multi-years experiments aiming at analyzing the response of AMF community to specific treatments.

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Statements and Declarations

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Competing Interests

The authors declare that they have no conflicts of interest.

Author Contributions

All authors conceived the study and experimental design. AKGD and NM collected samples. Material preparation, data collection, and analyses were performed by AKGD, who wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript and approved the final version.

Data Availability

We deposited the data generated during this study in the relevant publicly accessible databases as described in Material and Methods above.

Forest thinning has little effect on spatiotemporal dynamics of arbuscular mycorrhizal fungi community in *Cryptomeria japonica* roots

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Supplementary materials

Online Resource 1 Illustration of sampling design and microsites in the study site. MS, microsite. MS1 and MS4 were located at higher elevations compared to MS2 and MS5. Thinning occurred at microsites 2 and 5 but not at 1 and 4

Online Resource 2 Accumulation curves of operational taxonomic units (OTUs) of arbuscular mycorrhizal fungi (AMF) in *Cryptomeria japonica* roots before and after a thinning treatment

Online Resource 3 Phylogenetic tree of major operational taxonomic units (OTUs, average relative abundance > 1% at the study site) of arbuscular mycorrhizal fungi *Cryptomeria japonica* roots. A maximum likelihood tree was constructed using representative sequences of major OTUs (14 nucleotide sequences) and 44 reference nucleotide sequences downloaded from NCBI GenBank and Maarj*AM* databases. Aligned sequences had ~ 550 bp of the small subunit ribosomal DNA between the primer pairs NS31 and AM1 and covered 565 sites. The best model and parameters were selected with an automatic model finder in IQ-TREE 2. Ultrafast bootstrap (UFBoot) over 1000 randomizations were performed and UFboot ≥ 95% are shown at the nodes. Accessions of major OTUs and scientific names of reference sequences followed by their accessions were used for labeling

Table S1 Geographical location of microsites in the investigated artificial forest of *Cryptomeria japonica*
Five *C. japonica* trees were selected at each microsite. Values are presented as average (n = 5) ± SD for elevation which was measured at the base of each sampled tree. For the elevation, microsites with the same letter are not significantly different according to the analysis of variance followed by the Tukey's honestly significant difference test with Bonferroni p-value adjustment method

Table S2 Two-way mixed analysis of variance testing spatiotemporal variations in soil physicochemical properties at the study site
Significant effect reflected by p-value < 0.05. Pairwise comparisons are shown in Table S3

Table S3 Pairwise comparison of mean values of soil physicochemical properties between levels of microsite and sampling year
[1] For each variable, levels of the same factor with the same letter are not significantly different (Tukey's honestly significant difference test with Bonferroni p-value adjustment method, $p < 0.05$)

Table S4 Two-way mixed analysis of variance testing spatiotemporal variations in alpha diversity of arbuscular mycorrhizal fungi communities in *Cryptomeria japonica* roots

Significant effect reflected by *p*-value < 0.05. Pairwise comparisons are shown in Table S5

Table S5 Pairwise comparison of mean values of alpha diversity of arbuscular mycorrhizal fungi communities in *Cryptomeria japonica* roots between levels of microsite and sampling year

[1] For each variable, levels of the same factor with the same letter are not significantly different (Tukey's honestly significant difference test with Bonferroni p-value adjustment method , $p < 0.05$)

Table S6 Two-way nested permutational analysis of variance of arbuscular mycorrhizal fungi communities in *Cryptomeria japonica* roots

Significant effect reflected by *p*-value < 0.05. Pairwise comparisons for microsite are shown in Table S7

Table S7 Probabilities associated with multiple pairwise permutational analysis of variance of arbuscular mycorrhizal fungi communities in *Cryptomeria japonica* roots between levels of microsite

F- and *p*-values are shown above and below the diagonal of the table. p-value < 0.05 indicates significantly dissimilar assemblages of arbuscular mycorrhizal fungi between the microsites

Table S8 Correlation of soil physicochemichal properties with the composition of arbuscular mycorrhizal fungi communities in *Cryptomeria japonica* roots

Correlation and probability values were obtained by fitting environmental data to community data in redundancy analysis (RDA). RDA1 and RDA2 are the first and second axis of RDA, respectively. Significance is shown for each soil physicochemical property on the RDA plot (Fig. 2)

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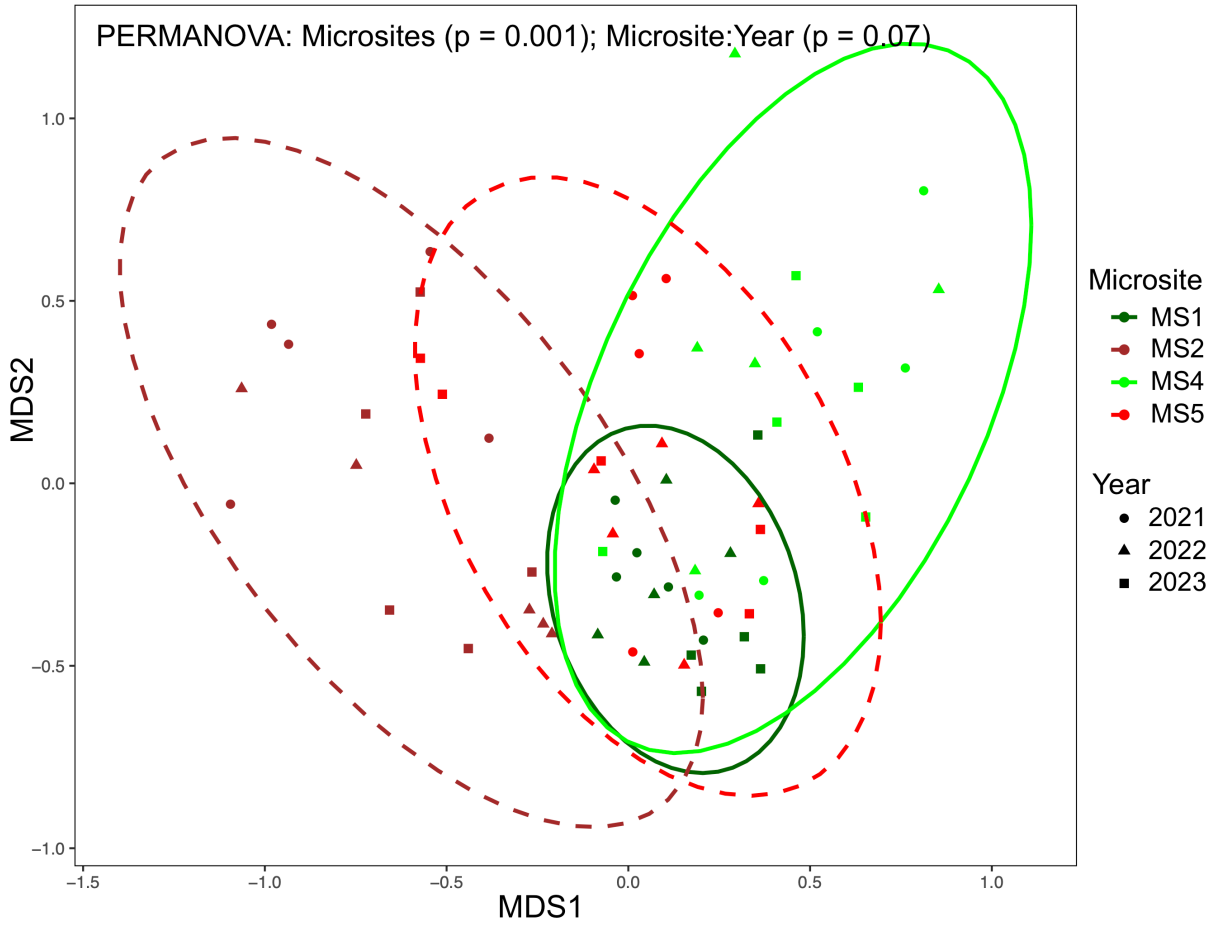

Fig. 1 Non-metric multidimensional scaling of the arbuscular mycorrhizal fungi (AMF) community *Cryptomeria japonica* roots at four microsites with and without thinning treatment. Stress = 0.20. Points represent samples, their color indicates the microsite, and their shape represents the sampling year. Thinning was applied in 2021 after sampling at MS2 and MS5 (dotted lines) but not at MS1 and MS4 (plain line). The AMF community was significantly different between microsites (PERMANOVA, $p = 0.001$) but not between sampling years at each microsite (PERMANOVA, $p = 0.07$)

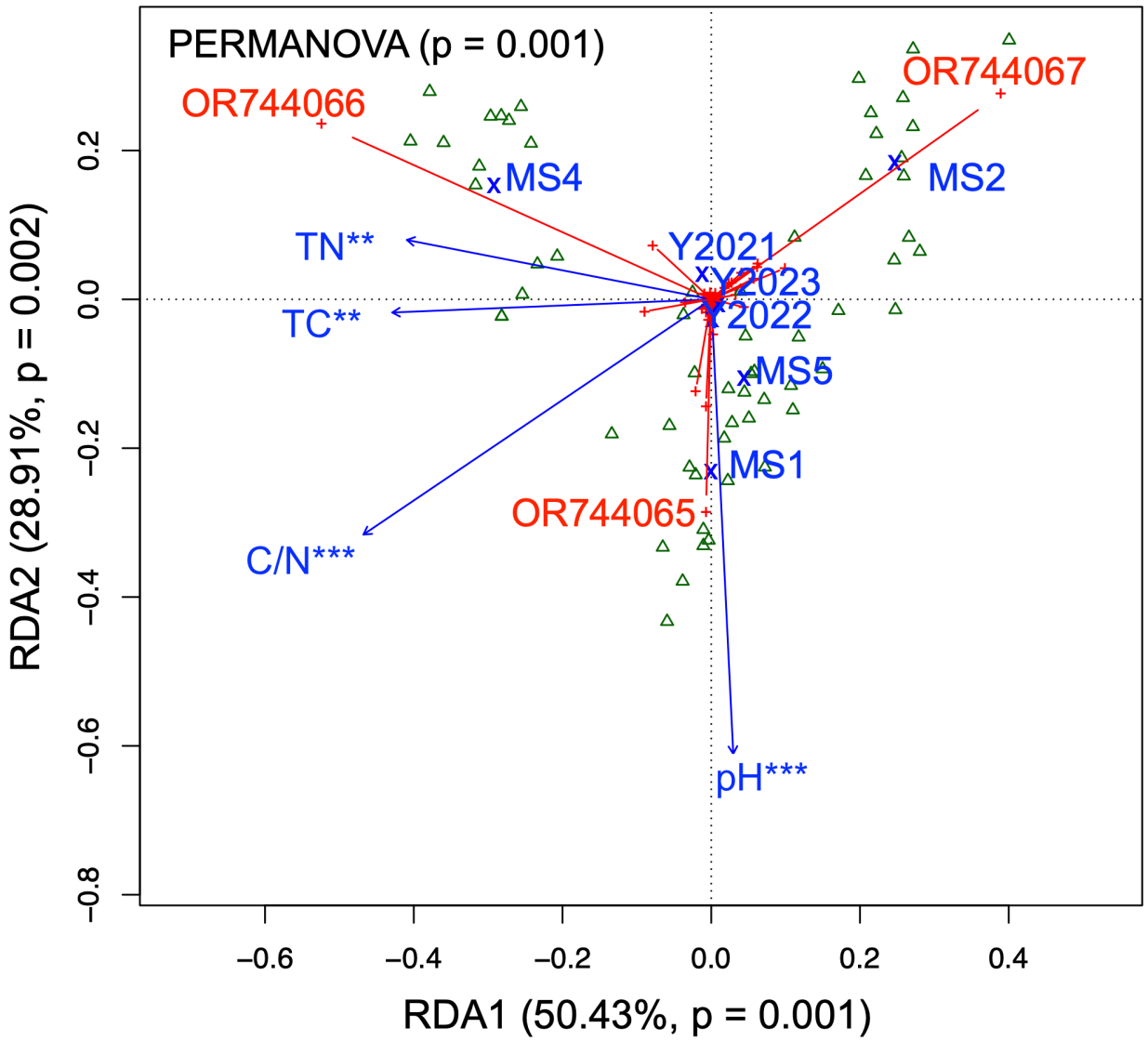

Fig. 2 Triplot of redundancy analysis (RDA) of the arbuscular mycorrhizal fungi (AMF) community in the roots of *Cryptomeria japonica*, visualized on scaling 2. Asterisks following the names of vectors indicate a significant correlation with the AMF community composition (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). OR###### are the accessions of the three operational taxonomic units (OTUs) most influenced by soil's physicochemical properties, explaining the variation observed in the AMF community; MS, microsite; TC, total C; TN, total N; C/N, carbon to nitrogen ratio

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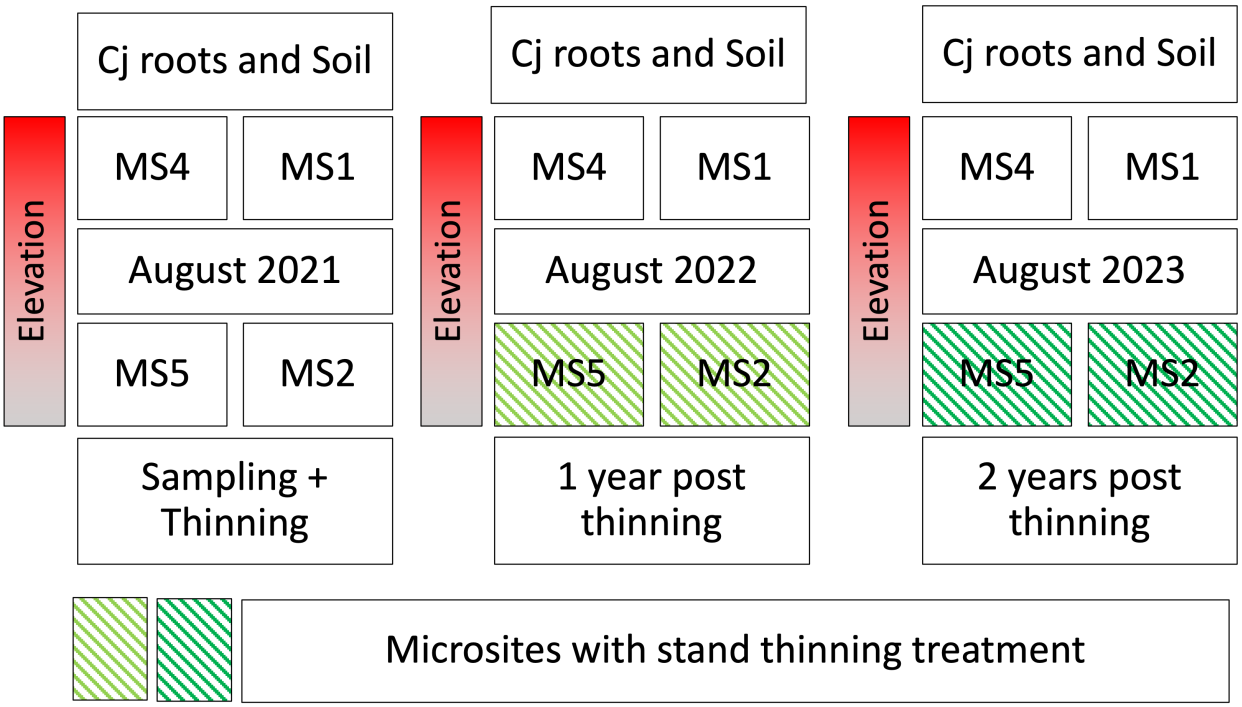

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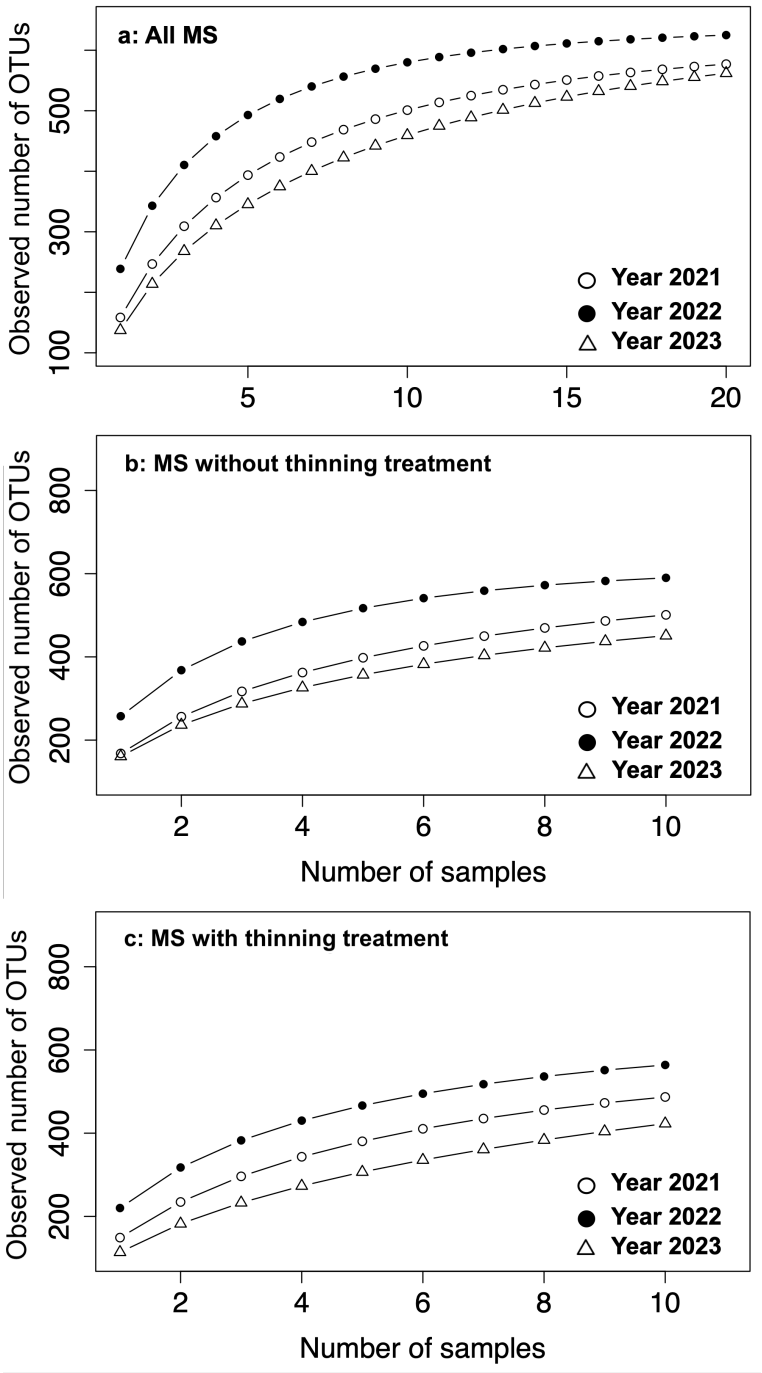

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577 mycorrhizal fungi in *Cryptomeria japonica* roots before and after a thinning treatment. Thinning
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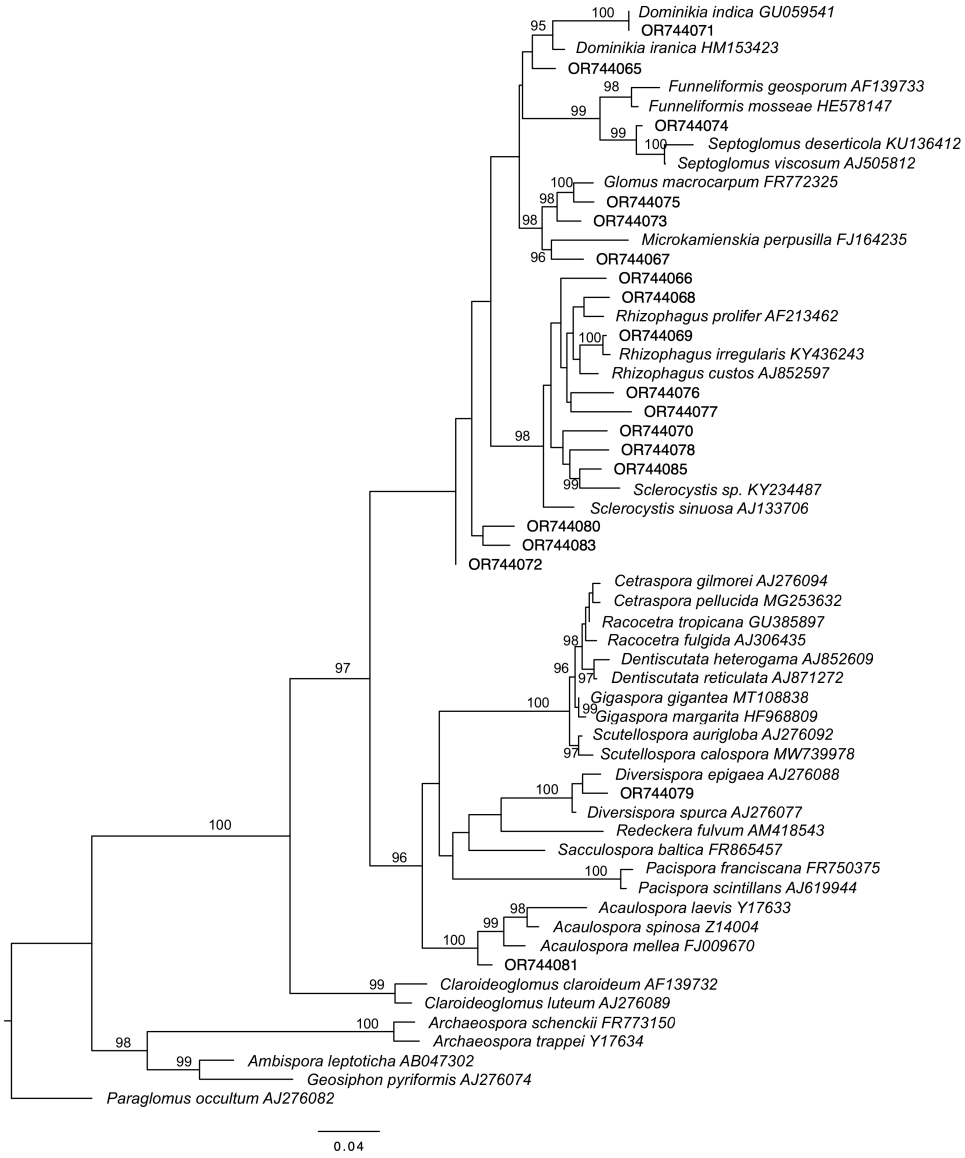

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Table 1 Soil physicochemical properties at four microsites established in an artificial *Cryptomeria japonica* forest

Microsites	Sampling year	pH	Total C (%)	Total N (%)	C/N
MS1	Y2021	5.7 ± 0.3	11.1 ± 2.1	0.7 ± 0.1	15.6 ± 0.5
	Y2022	5.9 ± 0.1	11.8 ± 3.2	0.7 ± 0.2	16.3 ± 1.1
	Y2023	5.9 ± 0.3	14.7 ± 3.7	0.9 ± 0.2	17.1 ± 1.0
MS2	Y2021	5.2 ± 0.4	12.7 ± 0.9	0.8 ± 0.0	15.0 ± 0.5
	Y2022	5.2 ± 0.3	11.3 ± 0.6	0.8 ± 0.0	15.0 ± 0.4
	Y2023	5.3 ± 0.2	14.5 ± 5.0	0.9 ± 0.2	15.9 ± 1.6
MS4	Y2021	5.6 ± 0.4	18.0 ± 3.3	1.1 ± 0.2	16.9 ± 0.4
	Y2022	5.0 ± 0.4	16.6 ± 2.4	1.0 ± 0.1	16.6 ± 0.5
	Y2023	5.0 ± 0.2	16.9 ± 1.8	1.0 ± 0.1	16.8 ± 0.3
MS5	Y2021	5.1 ± 0.2	17.3 ± 3.0	1.0 ± 0.1	16.5 ± 0.7
	Y2022	5.2 ± 0.2	16.4 ± 0.7	1.0 ± 0.0	16.7 ± 0.3
	Y2023	5.4 ± 0.3	19.4 ± 6.5	1.1 ± 0.2	17.5 ± 1.8

Five *C. japonica* trees were selected at each microsite. Values are presented as average (n = 5) ± SD for pH, total C, N, and C/N (carbon to nitrogen ratio). The interaction between microsite and sampling year was not significant (two-way mixed analysis of variance, $p > 0.05$). At a confidence level of 0.05, these soil's physicochemical properties were significantly different between microsites but not between sampling years, except for C/N (see Tables S2 and S3)

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Table 2 Alpha diversity of the arbuscular mycorrhizal fungi community in *Cryptomeria japonica* roots before and after stand thinning

Microsites	Sampling year		Number of operational taxonomic units (OTUs)	Shannon index
MS1	Y2021	-	181 ± 28	2.7 ± 0.2
	Y2022	-	285 ± 14	2.9 ± 0.1
	Y2023	-	146 ± 38	2.6 ± 0.2
MS2	Y2021	-	122 ± 18	2.2 ± 0.3
	Y2022	-	194 ± 44	2.5 ± 0.3
	Y2023	-	66 ± 09	2.3 ± 0.3
MS4	Y2021	-	155 ± 44	2.2 ± 0.4
	Y2022	-	229 ± 37	2.6 ± 0.6
	Y2023	-	176 ± 19	2.4 ± 0.3
MS5	Y2021	-	176 ± 23	2.5 ± 0.2
	Y2022	-	246 ± 37	2.9 ± 0.2
	Y2023	-	161 ± 29	2.6 ± 0.2

Five *C. japonica* trees were selected at each microsite. Values are presented as average (n = 5) ± SD. The interaction between microsite and sampling year was not significant (two-way mixed analysis of variance, $p > 0.05$). At a confidence level of 0.05, the number of OTUs and Shannon index were significantly different between microsites and sampling years (see Tables S4 and S5)

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Table 3 Distribution of the major operational taxonomic units of the arbuscular mycorrhizal fungi in the roots of *Cryptomeria japonica* before and after stand thinning

Dominant OTUs	Genus	Average relative abundance												
		MS1 (no thinning)			MS2 (thinning)			MS4 (no thinning)			MS5 (thinning)			(MS-Year)
		Y2021	Y2022	Y2023	Y2021	Y2022	Y2023	Y2021	Y2022	Y2023	Y2021	Y2022	Y2023	
OR744065*	*Dominikia*	0.328	0.266	0.205	0.298	0.210	0.274	0.304	0.233	0.271	0.097	0.132	0.175	0.233
OR744067*	*Microkamienskia*	0.053	0.218	0.033	0.049	0.170	0.012	0.021	0.006	0.042	0.327	0.027	0.156	0.093
OR744066*	unresolved Glomeraceae	0.030	0.007	0.235	0.023	0.000	0.031	0.066	0.351	0.044	0.014	0.244	0.013	0.088
OR744068*	*Rhizophagus*	0.054	0.031	0.042	0.053	0.044	0.148	0.025	0.058	0.085	0.070	0.046	0.071	0.061
OR744069*	*Rhizophagus*	0.062	0.010	0.164	0.040	0.051	0.018	0.053	0.077	0.038	0.028	0.032	0.081	0.055
OR744070	unresolved Glomeraceae	0.083	0.013	0.035	0.093	0.016	0.062	0.096	0.032	0.058	0.007	0.043	0.013	0.046
OR744071*	*Dominikia*	0.061	0.015	0.023	0.075	0.004	0.030	0.091	0.004	0.064	0.002	0.036	0.120	0.044
OR744072*	unresolved Glomeraceae	0.026	0.050	0.038	0.024	0.105	0.057	0.005	0.013	0.052	0.022	0.031	0.030	0.038
OR744078*	unresolved Glomeraceae	0.015	0.050	0.007	0.007	0.054	0.002	0.010	0.001	0.003	0.102	0.002	0.000	0.021
OR744075	*Glomus*	0.048	0.005	0.021	0.015	0.004	0.030	0.012	0.008	0.022	0.010	0.018	0.028	0.018
OR744076	*Rhizophagus*	0.011	0.002	0.014	0.004	0.002	0.051	0.034	0.023	0.022	0.011	0.028	0.013	0.018
OR744074	*Septoglomus*	0.001	0.021	0.003	0.023	0.013	0.019	0.020	0.014	0.040	0.001	0.013	0.031	0.017
OR744077	*Rhizophagus*	0.015	0.006	0.014	0.010	0.009	0.047	0.004	0.013	0.016	0.023	0.009	0.018	0.015
OR744073*	*Glomus*	0.003	0.010	0.015	0.002	0.000	0.000	0.004	0.001	0.002	0.001	0.136	0.004	0.015

OTUs, operational taxonomic units; MS, microsite. Dominant OTUs are marked with "*" and correspond to those with more than 10% of average relative abundance at one or more MS

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Forest thinning has little effect on spatiotemporal dynamics of arbuscular mycorrhizal fungi community in *Cryptomeria japonica* roots

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Table S1 Geographical location of microsites in the investigated artificial forest of *Cryptomeria japonica*

Microsites	Longitude (°E)	Latitude (°N)	Elevation (m)
MS1	138.8252064	35.94486857	1045.7 ± 6.6 a
MS2	138.825972	35.94276414	896.6 ± 11.4 c
MS4	138.8240636	35.94447363	1033.6 ± 6.1 a
MS5	138.8234634	35.94243657	909.5 ± 5.4 b

Five *C. japonica* trees were selected at each microsite. Values are presented as average (n = 5) ± SD for elevation which was measured at the base of each sampled tree. For the elevation, microsites with the same letter are not significantly different according to the analysis of variance followed by the Tukey's honestly significant difference test with Bonferroni *p*-value adjustment method

Table S2 Two-way mixed analysis of variance testing spatiotemporal variations in soil physicochemical properties at the study site

Effect	DFn	DFd	F	*p*	*p* < 0.05	Generalized effect size
pH						
Microsite (M)	3	14	17.50	< 0.001	*	0.62
Sampling year (S)	2	28	1.04	0.37		0.04
M:S	6	28	1.41	0.25		0.15
Total C						
M	3	14	8.68	< 0.01	*	0.41
S	1	16.95	3.50	0.07		0.14
M:S	4	16.95	0.35	0.82		0.05
Total N						
M	3	14	9.43	< 0.001	*	0.46
S	1	17.85	3.15	0.09		0.12
M:S	4	17.85	0.34	0.84		0.04
C/N						
M	3	14	7.42	< 0.001	*	0.37
S	1	17.3	4.97	0.03	*	0.18
M:S	4	17.3	0.54	0.70		0.07

Significant effect reflected by *p*-value < 0.05. Pairwise comparisons are shown in Table S3

Table S3 Pairwise comparison of mean values of soil's physicochemical properties between levels of microsite and sampling year

Soil's physicochemical properties	Levels of microsite	Number of replicates	Mean ± SD [1]	Significance of difference at $p < 0.05$
pH	MS1	15	5.9 ± 0.3 a	Significant
	MS2	15	5.2 ± 0.3 b	
	MS4	15	5.2 ± 0.4 b	
	MS5	15	5.2 ± 0.2 b	
Total carbon (C)	MS1	15	12.5 ± 3.3 b	Significant
	MS2	15	12.8 ± 3.1 b	
	MS4	15	17.2 ± 2.4 a	
	MS5	15	17.7 ± 4.0 a	
Total nitrogen (N)	MS1	15	0.8 ± 0.2 b	Significant
	MS2	15	0.8 ± 0.1 b	
	MS4	15	1.0 ± 0.1 a	
	MS5	15	1.0 ± 0.2 a	
C/N	MS1	15	16.3 ± 1.1 a	Significant
	MS2	15	15.3 ± 1.0 b	
	MS4	15	16.8 ± 0.4 a	
	MS5	15	16.9 ± 1.1 a	
Soil's physicochemical properties	Levels of sampling year	Number of replicates	Mean ± SD [1]	Significance of difference at $p < 0.05$
pH	Y2021	20	5.4 ± 0.4	Not significant
	Y2022	20	5.3 ± 0.4	
	Y2023	20	5.4 ± 0.4	
Total carbon (C)	Y2021	20	14.8 ± 3.8	Not significant
	Y2022	20	14 ± 3.2	
	Y2023	20	16.4 ± 4.7	
Total nitrogen (N)	Y2021	20	0.9 ± 0.2	Not significant
	Y2022	20	0.9 ± 0.2	
	Y2023	20	1.0 ± 0.2	
C/N	Y2021	20	16.0 ± 0.9 b	Significant
	Y2022	20	16.1 ± 0.9 ab	
	Y2023	20	16.8 ± 1.4 a	

[1] For each variable, levels of the same factor with the same letter are not significantly different (Tukey's honestly significant difference test with Bonferroni p-value adjustment method, $p < 0.05$)

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Table S4 Two-way mixed analysis of variance testing spatiotemporal variations in alpha diversity of arbuscular mycorrhizal fungi communities in *Cryptomeria japonica* roots

Effect	DFn	DFd	F	*p*	$p < 0.05$	Generalized effect size
Number of operational taxonomic units (OTUs)						
Microsite (M)	3	16	24.38	< 0.001	*	0.55
Sampling year (S)	2	32	55.40	< 0.001	*	0.72
M:S	6	32	2.32	0.06		0.24
Shannon index						
M	3	16	12.48	< 0.001	*	0.33
S	2	32	4.74	0.02	*	0.19
M:S	6	32	0.18	0.98		0.03

Significant effect reflected by *p*-value < 0.05. Pairwise comparisons are shown in Table S5

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Table S5 Pairwise comparison of mean values of alpha diversity of arbuscular mycorrhizal fungi communities in *Cryptomeria japonica* roots between levels of microsite and sampling year

Alpha diversity index	Levels of microsite	Number of replicates	Mean ± SD [1]	Significance of difference at $p < 0.05$
Number of operational taxonomic units (OTUs)	MS1	15	204 ± 67 a	Significant
	MS2	15	127 ± 60 b	
	MS4	15	187 ± 46 a	
	MS5	15	195 ± 48 a	
Shannon index	MS1	15	2.8 ± 0.2 a	Significant
	MS2	15	2.3 ± 0.3 b	
	MS4	15	2.4 ± 0.5 b	
	MS5	15	2.7 ± 0.2 a	
Alpha diversity index	Levels of sampling year	Number of replicates	Mean ± SD [1]	Significance of difference at $p < 0.05$
Number of operational taxonomic units (OTUs)	Y2021	20	158 ± 37 b	Significant
	Y2022	20	239 ± 47 a	
	Y2023	20	137 ± 50 b	
Shannon index	Y2021	20	2.4 ± 0.4 b	Significant
	Y2022	20	2.7 ± 0.4 a	
	Y2023	20	2.5 ± 0.3 ab	

[1] For each variable, levels of the same factor with the same letter are not significantly different (Tukey's honestly significant difference test with Bonferroni p-value adjustment method , $p < 0.05$)

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Table S6 Two-way nested permutational analysis of variance of arbuscular mycorrhizal fungi communities in *Cryptomeria japonica* roots

Effect	Df	SumOfSqs	R2	*F*	Pr(>F)
Microsite (M)	3	3.0913	0.27	7.2477	< 0.001
M:Sampling year	8	1.461	0.13	1.28	0.07
Residual	48	6.8245	0.60		
Total	59	11.3768	1.00		

Significant effect reflected by *p*-value < 0.05. Pairwise comparisons for microsite are shown in Table S7

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Table S7 Probabilities associated with multiple pairwise permutational analysis of variance of arbuscular mycorrhizal fungi communities in *Cryptomeria japonica* roots between levels of microsite

Microsites	MS1	MS2	MS4	MS5
MS1		9.92	7.32	2.53
MS2	< 0.001		10.03	6.36
MS4	< 0.001	< 0.001		5.02
MS5	0.01	< 0.001	< 0.001	

F- and *p*-values are shown above and below the diagonal of the table. *p*-value < 0.05 indicates significantly dissimilar assemblages of arbuscular mycorrhizal fungi between the microsites

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Table S8 Correlation of soil physicochemichal properties with the composition of arbuscular mycorrhizal fungi communities in *Cryptomeria japonica* roots

Soil physicochemiacal property	RDA1	RDA2	r^2	Pr(>r)	Significance
pH	0.001	-1.000	0.261	0.001	***
Total C	-0.997	-0.075	0.196	0.003	**
Total N	-0.997	0.082	0.179	0.004	**
C/N	0.001	-1.000	0.261	0.001	***

Correlation and probability values were obtained by fitting environmental data to community data in redundancy analysis (RDA). RDA1 and RDA2 are the first and second axis of RDA, respectively. Significance is shown for each soil physicochemical property on the RDA plot (Fig. 2)

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